6.25
Arrange weekly equipment preparation.
Week1 Hu Changxing
Week2 Shu Fang
Week3 Yi Dawei
Week4 Liao Yuxia
Week5 Zhu Shanshan
Week6 Tao Xumin
And so on

6.26
Weekly equipment preparation (On duty:Hu Changxing):

EP tube(sterilized、unsterilized)、PCR tube (sterilized、unsterilized)、Pipette tip(sterilized、unsterilized)

Prepare LB liquid medium
Prepare 1×TAE solution
Clean up the Lab

6.27
Design primer BD CRY2 CIB1

Ask biological company to synthetize AD-UAS 、Pre-amiRNA-D1、Pre-amiRNA-FNR

Prepare sterile water
Buy PCR enzyme
People in lab: Hu Changxing, Shu Fang
6.30

Receive PCR enzyme、BD CRY2 CIB1 primer

PCR Gal BD：use pGBKT7 as template

PCR CRY2 and CIB1：use Arabidopsis thaliana's cDNA as template

Prepare glue

Glue leaking——failed analyze：we made some mistakes when designing primers, he difference of annealing temperature between upstream and downstream primers is too big.
Redesign BD primer
People in lab:Hu Changxing Shu Fang Yi Dawei

7.1
Receive new BD primer

PCR Gal BD：use pGBKT7 as template

PCR CRY2 and CIB1：use Arabidopsis thaliana's cDNA as template

Prepare glue

Glue leaking——failed　　analyze：PCR tube's lid sprang open when pcr,a part of the solution is evaporated.
People in lab:Hu Changxing　Shu Fang　Yi Dawei

7.3
Weekly equipment preparation(On duty:Shu Fang)
Supplement the pipette tip(unsterilized)and PCR tube(unsterilized)
Prepare LB agar
Buy T-Vector and competent cells
Prepare sterilized toothpick
Clean up the Lab

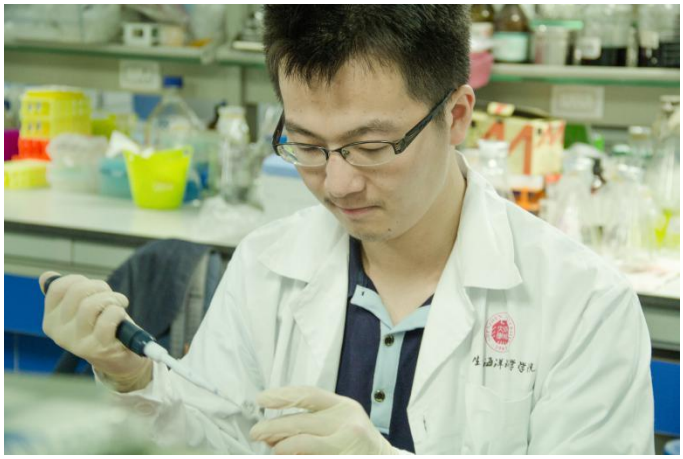
7.4

PCR BD CIB1 CRY2
Prepare glue
Glue leaking——succeed
Gel extraction

Concentration measurement——failed the concentration is too low analyze :the

temperature for glue melting is too high ， DNA was degraded .

People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan



7.5
PCR: BD CIB1 CRY2
Prepare glue
Glue leaking——succeed
Gel extraction
Concentration measurement——BD 263.7ng/μL CRY2 218.3ng/μL, CIB1 107.3ng/μL
People in lab: Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan

7.6
Receive T-Vector and competent cells
Connect BD CIB1 CRY2 to T-Vector
Transform BD CIB1 CRY2 to competent cells
Plate sample on LB agar
People in lab: Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan

7.7

No colonies grow on agar medium

analyze：we use LB(+Amp)to shake bacteria fluid after transformation，it is wrong，

we should use LB(-Amp)instead.

Connect BD CIB1 CRY2 to T-Vector

Transform BD CIB1 CRY2 to competent cells

Plate sample on LB agar

People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

7.8

Too many colonies grow on agar medium analyze：too late to pick out the plate medium.

Connect BD CIB1 CRY2 to T-Vector

Transform BD CIB1 CRY2 to competent cells

Plate sample on LB agar

People in lab: Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

7.9

Colonies grow well on agar medium

Select monoclonal colonies，3 for each plate

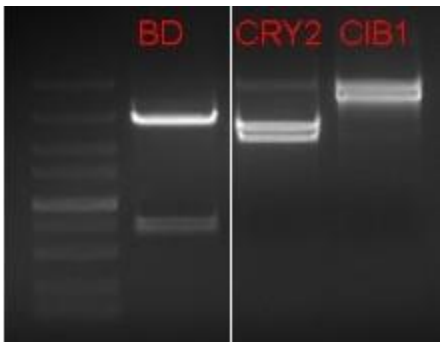
shake bacteria fluid for 5h
Sequencing
People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

7.10
Weekly equipment preparation(On duty:Yi Dawei)

Supplement the pipette tip(sterilized、unsterilized)and PCR tube(sterilized、unsterilized)
Prepare LB liquid medium
Prepare LB agar
Clean up the Lab

7.12

Sequencing result：no positive result analyze：we took too long time to cut glue under ultraviolet rays
PCR BD CIB1 CRY2
Prepare glue
Glue leaking——succeed



Gel extraction
Concentration measurement——BD 159.7ng/μL CRY2 124.2ng/μL CIB1 130.1ng/μL
People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

7.13
Connect BD CIB1 CRY2 to T-Vector
Transform BD CIB1 CRY2 to competent cells
Plate sample on LB agar

People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

7.14
Colonies grow well on agar medium

Select monoclonal colonies，4 for each plate

shake bacteria fluid for 5h
Sequencing
People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

7.17
Weekly equipment preparation(On duty:Liao Yuxia)

Supplement the pipette tip(sterilized、unsterilized)and PCR tube(sterilized、unsterilized)
Prepare LB agar
Clean up the Lab

7.18

Sequencing result：positive：BD③④ CIB1② CRY2①

shake bacteria fluid BD③ CIB1② CRY2①
People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

7.19
Plasmid extraction
Concentration measurement——BD 127.3ng/μL, CRY2 120.2ng/μL CIB1 130.7ng/μL
People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

7.20

Enzyme digestion AD-UAS、amiRNA-D1、amiRNA-FNR

Prepare glue

Glue leaking——failed　　analyze：the time for enzyme digestion is too short.

People in lab:Hu Changxing　Shu Fang　Yi Dawei　Liao Yuxia　Zhu Shanshan　Tao Xumin

7.21

Enzyme digestion AD-UAS、amiRNA-D1、amiRNA-FNR

Prepare glue

Glue leaking——failed　　analyze：the amount of enzyme is too low

People in lab:Hu Changxing　Shu Fang　Yi Dawei　Liao Yuxia　Zhu Shanshan　Tao Xumin

7.22

Enzyme digestion AD-UAS、amiRNA-D1、amiRNA-FNR

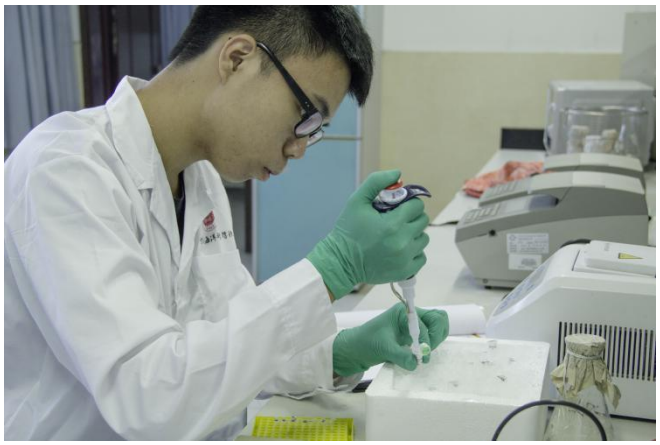
Prepare glue

Glue leaking——AD-UAS failed　　analyze：there are a methylation on the restriction site of AD-UAS

Gel extraction

Buy demethylation competent cell

People in lab:Hu Changxing　Shu Fang　Yi Dawei　Liao Yuxia　Zhu Shanshan　Tao Xumin



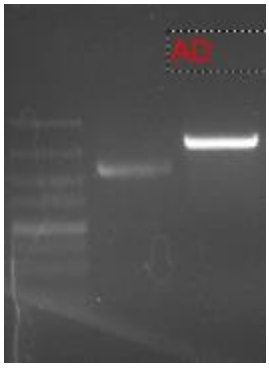
7.24
Weekly equipment preparation(On duty:Zhu Shanshan)

Supplement the pipette tip(sterilized、unsterilized)and PCR tube(sterilized、unsterilized)
Prepare TAP liquid medium

Prepare TAP agar(+Paro、+Paro and Hyg)

Clean up the Lab

7.28
Receive demethylation competent cell
Enzyme digestion AD-UAS
Prepare glue
Glue leaking——succeed



Gel extraction

People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

7.29
Weekly equipment preparation(On duty:Tao Xunmin)

Supplement the pipette tip(sterilized、unsterilized)and PCR tube(sterilized、

unsterilized)
Prepare LB liquid medium
Clean up the Lab

8.1

Gene ligation——CRY2、AD-UAS、amiRNA-D1、pDh124

CRY2、AD-UAS、amiRNA-FNR、pDh124

CIB1、BD、pDp124

Prepare glue
Glue leaking——failed
People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

8.2

Gene ligation——CRY2、AD-UAS、amiRNA-D1、pDh124

CRY2、AD-UAS、amiRNA-FNR、pDh124

CIB1、BD、pDp124

Prepare glue
Glue leaking——failed
People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

8.3

Gene ligation——CRY2、AD-UAS、amiRNA-D1、pDh124

CRY2、AD-UAS、amiRNA-FNR、pDh124

CIB1、BD、pDp124

Prepare glue
Glue leaking——succeed
People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

8.4
Weekly equipment preparation(On duty:Hu Changxing)

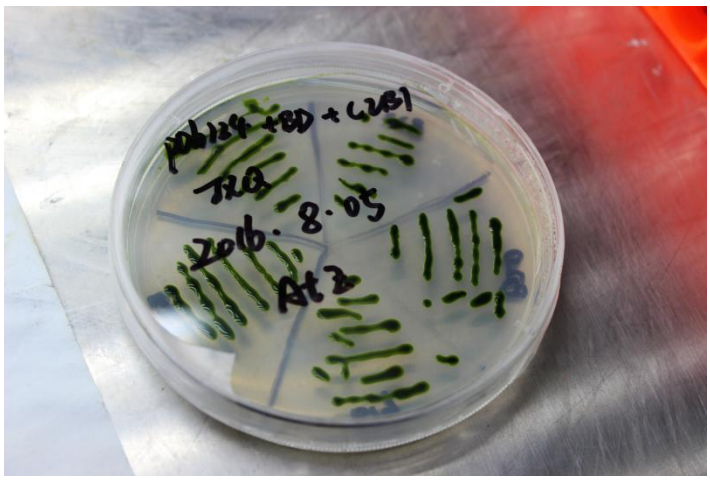
EP tube(sterilized、unsterilized)、PCR tube (sterilized、unsterilized)、Pipette tip(sterilized、unsterilized)

Prepare TAP liquid medium
Prepare TAP agar(+Paro, +Paro and Hyg)
Clean up the Lab

8.5
Transform pDp124-BD-CIB1 into Chlamydomonas reinhardtii by glass bead method day1
People in lab:Hu Changxing Shu Fang Liao Yuxia Zhu Shanshan

8.6
Transform pDp124-BD-CIB1 into Chlamydomonas reinhardtii by glass bead method day2
People in lab:Hu Changxing Shu Fang Liao Yuxia Zhu Shanshan

8.21
Pick positive transformants and put them into liquid TAP medium.
People in lab:Hu Changxing



8.25
Weekly equipment preparation(On duty:Shu Fang)
Prepare TAP liquid medium
Clean up the lab

8.27
DNA extraction of the transformants
People in lab:Hu Changxing　Shu Fang

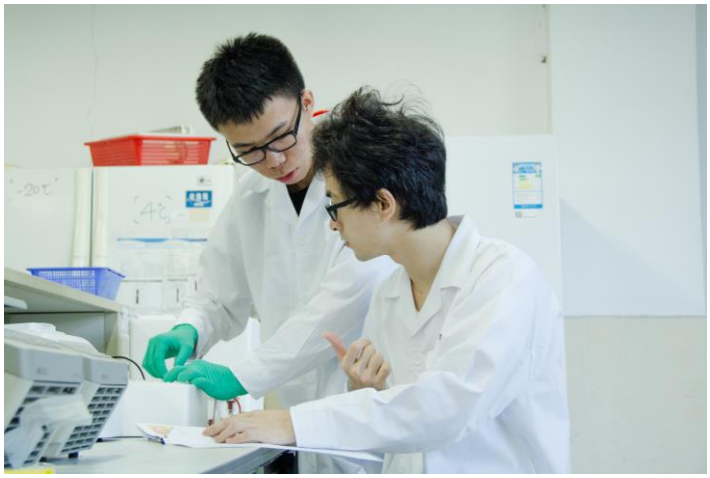
8.28
Weekly equipment preparation(On duty:Yi Dawei)

EP tube(sterilized、unsterilized)、PCR tube (sterilized、unsterilized)、Pipette tip(sterilized、unsterilized)

Prepare TAP liquid medium
Clean up the lab

8.29
PCR BD CIB1
Prepare glue
Glue leaking——failed
People in lab:Hu Changxing　Shu Fang　Liao Yuxia　Zhu Shanshan

8.30
PCR BD CIB1
Prepare glue
Glue leaking——failed
People in lab:Hu Changxing　Shu Fang　Liao Yuxia　Zhu Shanshan



8.31
PCR BD CIB1
Prepare glue

Glue leaking——failed　　analyze：the quality of DNA we extract is not good

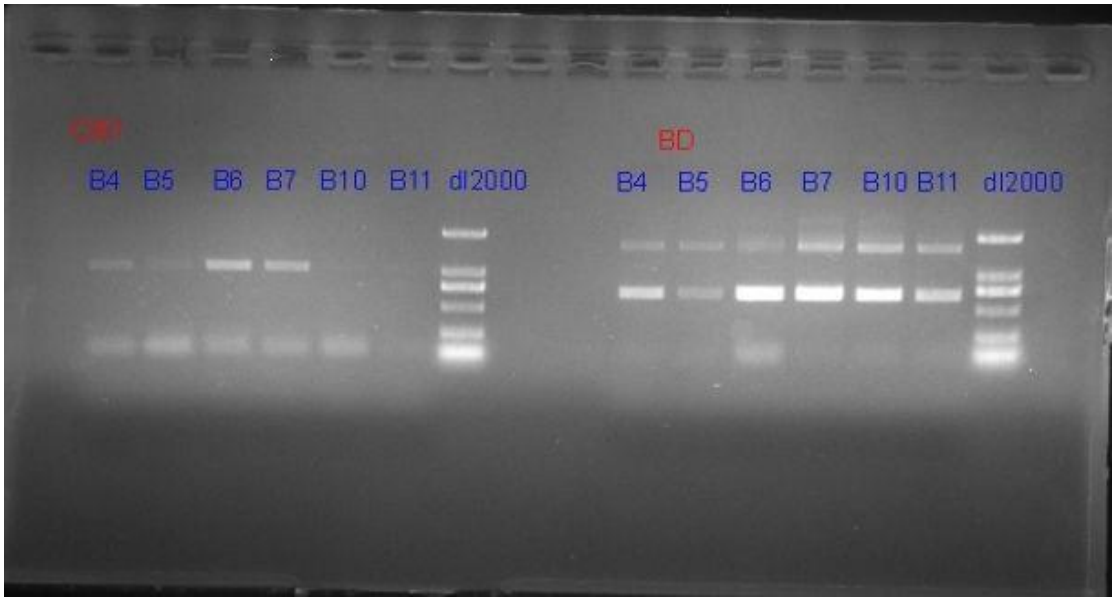
People in lab:Hu Changxing　Shu Fang　Liao Yuxia　Zhu Shanshan

9.4
Weekly equipment preparation(On duty:Liao Yuxia)

Supplement the pipette tip(sterilized、 unsterilized)and PCR tube(sterilized、 unsterilized)
Prepare TAP liquid medium
Prepare TAP agar(+Paro and Hyg)
Clean up the lab

9.5
DNA extraction of the transformants
People in lab:Hu Changxing　Shu Fang　Liao Yuxia　Zhu Shanshan

9.6
PCR BD CIB1
Prepare glue
Glue leaking——succeed



Gel extraction
sequencing
People in lab:Hu Changxing Shu Fang Liao Yuxia Zhu Shanshan

9.10

Sequencing result：positive CIB1⑥⑦ BD⑥⑦⑩

So ⑥and⑦ are positive transformants
People in lab:Hu Changxing Shu Fang Liao Yuxia Zhu Shanshan

9.11
Weekly equipment preparation(On duty:Zhu Shanshan)

Supplement the pipette tip(sterilized、 unsterilized)and PCR tube(sterilized、 unsterilized)
Prepare TAP liquid medium
Prepare TAP agar(+Paro and Hyg)
Clean up the lab

9.12
Transform pDh124-VP16-CRY2-UAS into pDp124-BD-CIB1 transformant⑥by glass bead method day1
People in lab:Hu Changxing Shu Fang Liao Yuxia Zhu Shanshan

9.13
Transform pDh124-VP16-CRY2-UAS into pDp124-BD-CIB1 transformant⑥by glass bead method day2
People in lab:Hu Changxing Shu Fang Liao Yuxia Zhu Shanshan

9.14
Prepare TAP liquid medium
Prepare TAP agar(+Paro and Hyg)
People in lab:Liao Yuxia　Zhu Shanshan

9.18
Weekly equipment preparation(On duty:Tao Xumin)

Supplement the pipette tip(sterilized、unsterilized)and PCR tube(sterilized、unsterilized)

9.21
Pick positive transformants and put them into liquid TAP medium.
People in lab:Hu Changxing　Shu Fang　Liao Yuxia　Zhu Shanshan

9.25
Weekly equipment preparation (On duty: Hu Changxing)
Prepare TAP liquid medium

9.28
DNA extraction of the transformants
People in lab:Hu Changxing　Shu Fang　Liao Yuxia　Zhu Shanshan

9.29
PCR AD-UAS CRY2 amiRNA-D1 amiRNA-FNR
Prepare glue
Glue leaking——failed
People in lab: Hu Changxing　Shu Fang　Liao Yuxia , Zhu Shanshan

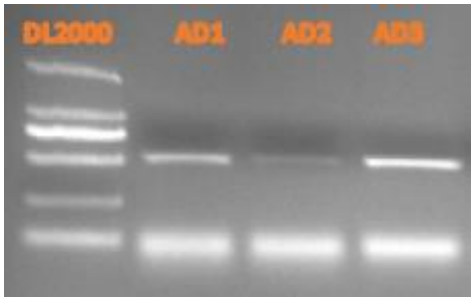
9.30

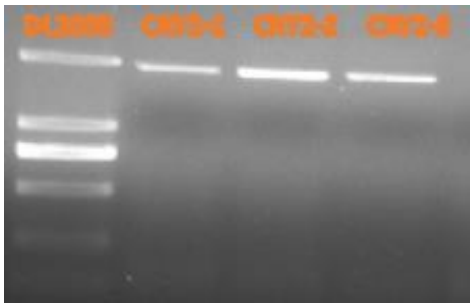
PCR AD-UAS CRY2 amiRNA-D1 amiRNA-FNR

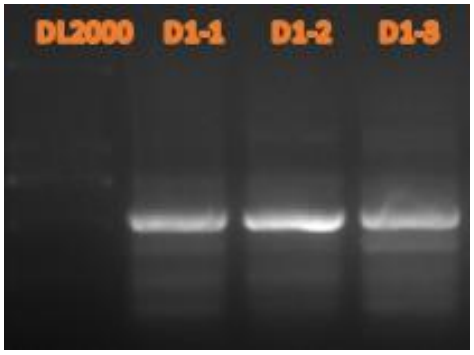
Prepare glue

Glue leaking——succeed

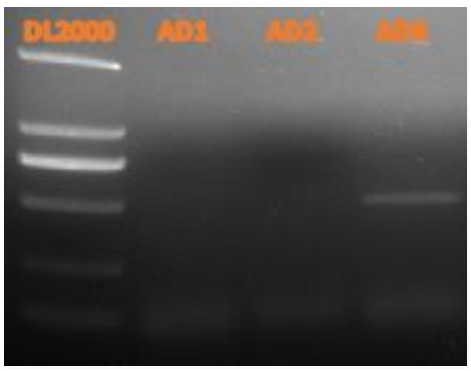
D1 transformants：

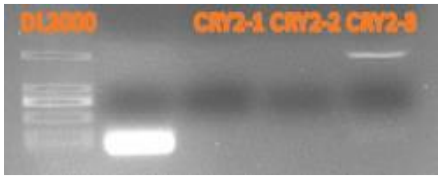


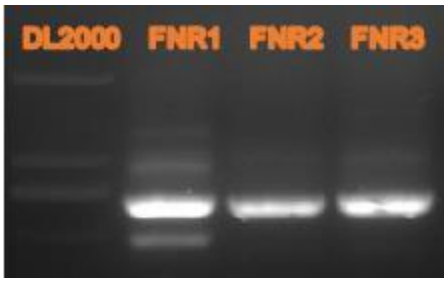




FNR transformants：







Gel extraction
Sequencing
People in lab:Hu Changxing　Shu Fang　Liao Yuxia　Zhu Shanshan

10.2
Weekly equipment preparation(On duty:Shu Fang)

EP tube(sterilized、unsterilized)、PCR tube (sterilized、unsterilized)、Pipette tip(sterilized、unsterilized)

Prepare 1×TAE solution
Clean up the Lab

10.3

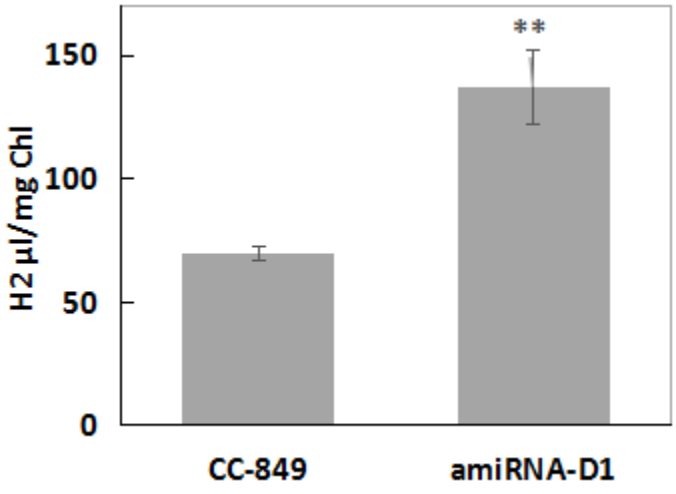
Sequencing result：positive D1① FNR③

So D1①and FNR③ are positive transformants
Grow D1①and FNR③ in 250mL TAP liquid medium.

10.7
The algae reached logarithmic phase
Begin the detection of hydrogen yield
We cultivated the gene-modified green algae in blue light (The wave length is about 450 nm) and induced the expression of relevant miRNA.
People in lab:Hu Changxing　Shu Fang　Yi Dawei　Liao Yuxia　Zhu Shanshan　Tao Xumin

10.10
The algae reached logarithm stage, we measured hydrogen production.

Result:



People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

10.12

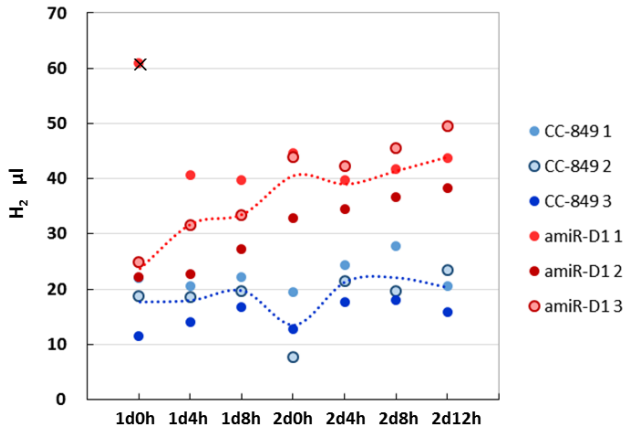
We cultivated the gene-modified green algae in the condition lacking in blue light. (Red light LED plant growth lamp, peak wave length: 660nm, half width: 17.9nm, dominant wave length: 642.4 nm, purity: 0.989).

People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin

10.15

The green algae reached logarithm stage, we used blue light, whose wave length is about 450 nm, to induce the expression of the relevant miRNA and increase hydrogen production. First we used blue light to induce the expression of miRNA and measured hydrogen production every 4 hours. After 8 hours, we re-cultivated the green algae in red light. After 16 hours, again we used blue light to induce the expression of miRNA and measured hydrogen production every 4 hours for 12 hours.

Result:



People in lab:Hu Changxing Shu Fang Yi Dawei Liao Yuxia Zhu Shanshan Tao Xumin